#9

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Blatt, et al.

Serial No.: 09/740,332

Filed: December 18, 2000

For: ENZYMATIC NUCLEIC ACID TREATMENT OF DISEASES OR CONDITIONS RELATED TO HEPATITIS C VIRUS INFECTION

Group Art Unit:

Examiner: not assigned

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant respectfully request that this amendment be entered prior to examination of the above-mentioned application on the merits. It is believed that no fee is due for filing this response; however, if a fee is due, the Commissioner is authorized to charge our Deposit Account No. 132490.

IN THE SPECIFICATION:

On page 1, after the first paragraph, please insert the following new paragraph:

" The Sequence Listing file named "MBHB00-801-F Sequence Listing.txt" submitted on Compact Disc-Recordable (CD-R) medium ("010326_2122") in compliance with 37 C.F.R. §1.52(e) is incorporated herein by reference."

CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington D.C. 20231, on March 26, 2001.

Date: March 7, 2001

Anita J. Terpstra

On page 15, please replace paragraph 2 with the following substitute paragraph:

" By "consists essentially of" is meant that the active enzymatic nucleic acid molecule of the invention contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind RNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage. Thus, a core region may, for example, include one or more loop or stem-loop structures, which do not prevent enzymatic activity. Such sequences can be designated as "X", for example, as in a loop or stem/loop structure. For example, a core sequence for a hammerhead enzymatic nucleic acid can be 5'- CUGAUGAG -3' and 5' - CGAA -3' connected by "X", where X= 5' - GCCGUUAGGC -3' (SEQ ID NO: 9704), or any other stem II region known in the art. Similarly, for other enzymatic nucleic acid molecules of the instant invention, additional sequences may be present that do not interfere with the function of the nucleic acid molecule."

Please replace originally-filed pages 69-72 with the substitute pages 69-72 submitted herewith.

IN THE ABSTRACT

On page 176, please replace the originally-filed abstract with the following substitute abstract:

"The present invention relates to enzymatic nucleic acid molecules (e.g., ribozymes and DNAzymes) that modulate the expression and/or replication of hepatitis C virus (HCV)."

IN THE FIGURES:

Please replace Figures 1-5, 7 and 8 with substitute Figures 1-5, 7 and 8, submitted herewith.

SEQUENCE LISTING:

Please incorporate by reference the sequence listing submitted herewith. The sequences in the sequence listing are found in the originally-filed specification and figures.

REMARKS

Specification

Page 1 of the specification was amended to incorporate by reference the sequence listing, submitted herewith.

Page 15, paragraph 2, of the specification was amended to provide clarification and to include the appropriate SEQ ID NO, in compliance with 37 C.F.R § 1.821-1.825.

Pages 69-72 of the specification were amended to provide the appropriate reference numbers for the references cited in Table 1.

No new matter has been added by way of any of these amendments to the specification.

A copy of the marked up original sheets for the described pages are attached as Appendix A and Appendix B.

Abstract

The abstract was amended merely to provide clarification. A copy of the marked up original abstract is attached as Appendix C.

Figures

New Figures 1-5, 7 and 8 were amended to include the appropriate SEQ ID NOs in compliance with 37 C.F.R § 1.821-1.825. No new matter has been added by way of these amendments.

A marked-up version of the original Figures 1-5, 7 and 8 are attached as Appendix D.

Sequence Listing

A sequence listing is attached which includes the sequences found in the specification and drawings. In compliance with 37 C.F.R § 1.821-1.825 and § 1.52(e), the applicant herewith submits the Sequence Listing on Compact Disc-Recordable (CD-R) medium in duplicate (COPY 1 and COPY 2), in lieu of the paper copy under 37 C.F.R. § 1.821(c), and substitute computer readable form copy (COPY 3). The paper and computer readable forms of the Sequence Listing are the same. The Statements under 37 CFR § 1.821(f) and § 1.52(e) are also provided.

The Sequence Listing has been generated from the specification and does not constitute new subject matter. The Sequence Listing has been prepared in the PatentIn Ver.2.0 format and checked with Checker Version 3.0 Program. No error has been found.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

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Appendix A

By "consists essentially of" is meant that the active enzymatic nucleic acid molecule of the invention contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind RNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage. Thus, a core region may, for example, include one or more loop or stem-loop structures, which do not prevent enzymatic activity. Such sequences can be designated as "X", for example, as in a loop or stem/loop structure. For example, a [A] core sequence for a hammerhead enzymatic nucleic acid can be 5'- CUGAUGAG -3' [X] and 5' - CGAA -3' connected by "X", where X= 5' - GCCGUUAGGC -3' (SEQ ID NO: 9704), or any other stem II region known in the art. Similarly, for other enzymatic nucleic acid molecules of the instant invention, additional sequences may be present that do not interfere with the function of the nucleic acid molecule.

Appendix B

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [xxxix].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [xl].
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [xli]

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xli Puttaraju, M.; Perrotta, Anne T.; Been, Michael D.. A circular trans-acting hepatitis delta virus ribozyme. Nucleic Acids Res. (1993), 21(18), 4253

Appendix C

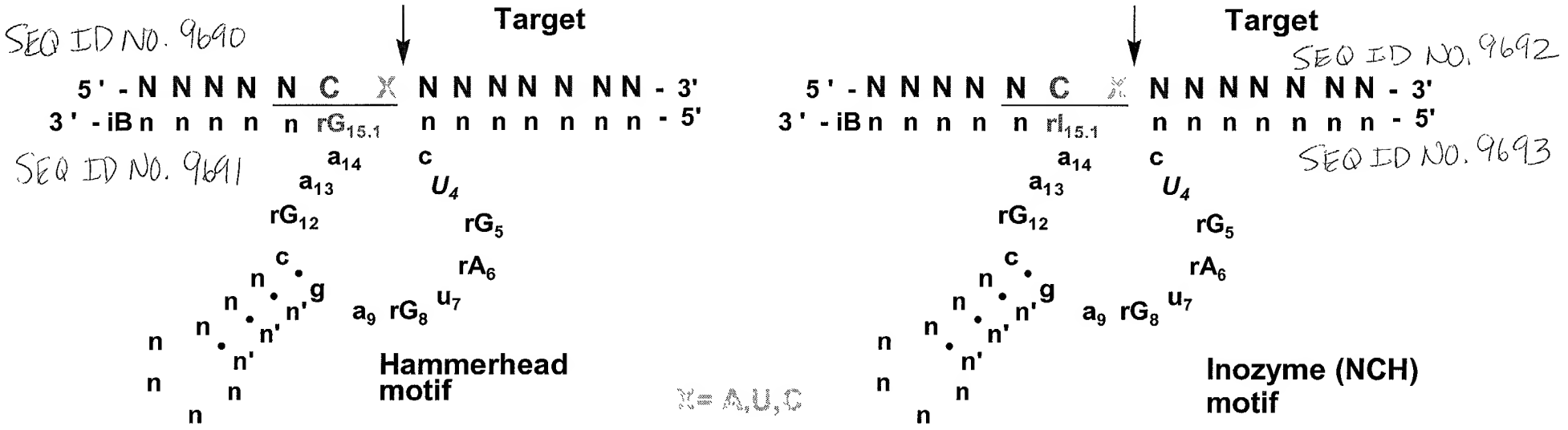
Abstract of the Disclosure

The present invention relates to [E]enzymatic nucleic acid molecules (e.g., ribozymes and DNAzymes) that modulate the expression and/or replication of hepatitis C virus (HCV).

Appendix D

20820" 2EOn260

Figure 1: Examples of Chemically Stabilized Enzymatic Nucleic Acid motifs



Lower case = 2'-*O*-Me

rN = ribonucleotide

U_4 = 2'-*C*-Allyl

rI = ribo Inosine modification

iB = inverted deoxy abasic

Figure 2: G-cleaver Motif

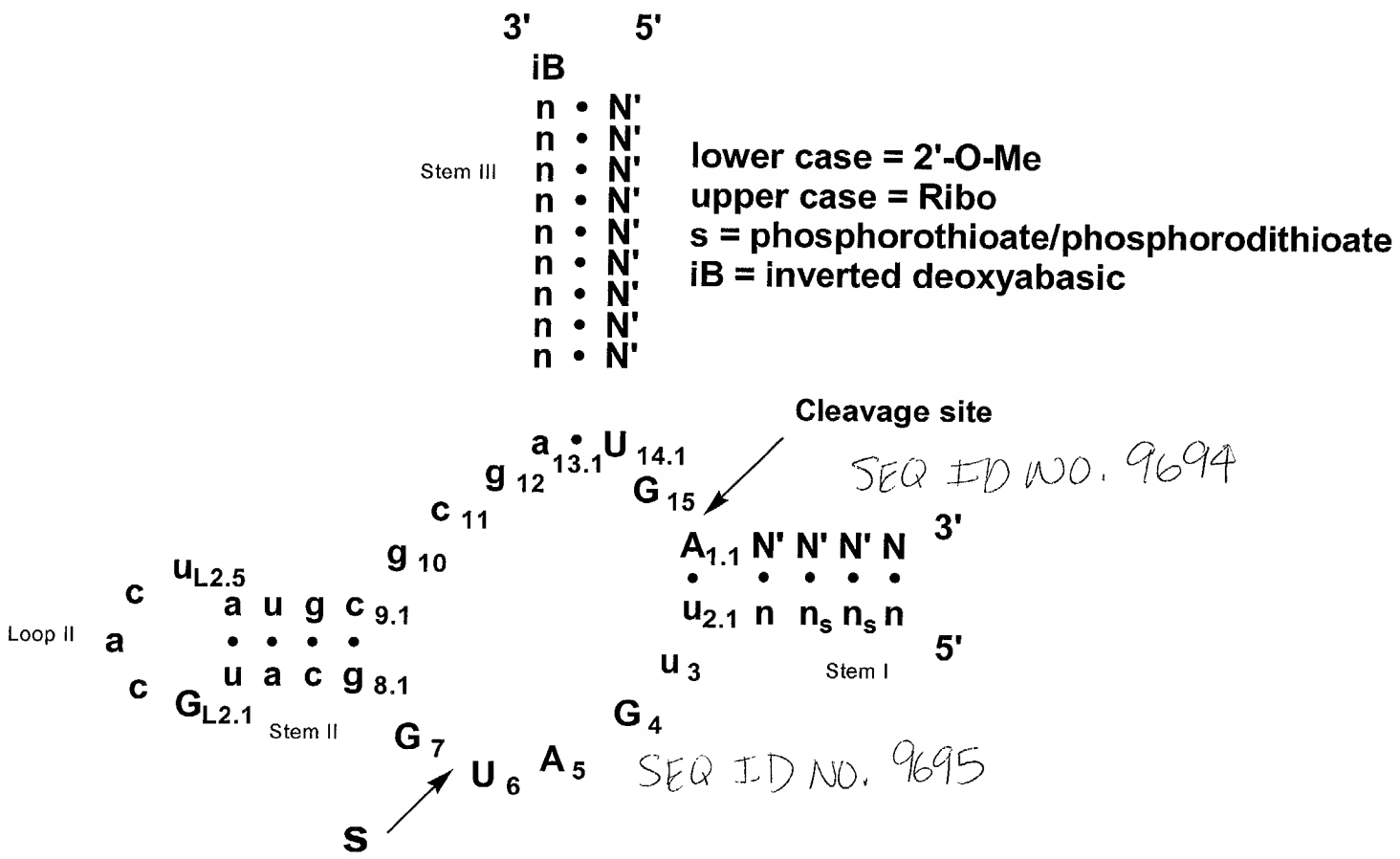
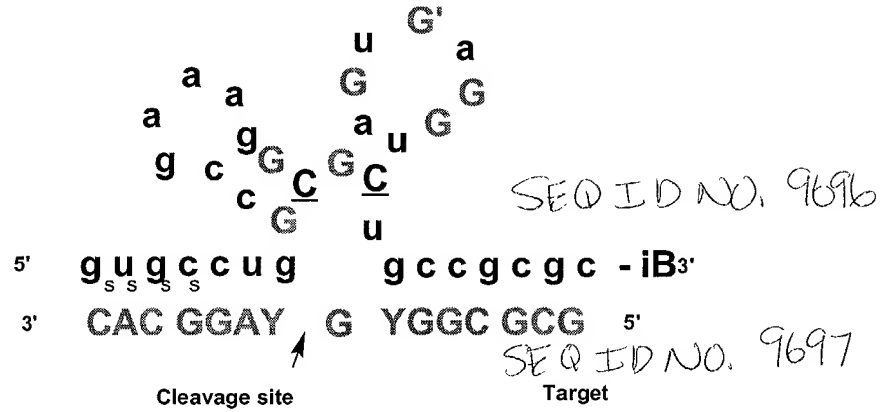


Figure 3: Chemically Stabilized Zinzyme Motif



Legend

Uppercase indicates natural ribo residues

C indicates 2'-deoxy-2'-amino cytidine

Lowercase: 2'-*O*- Me

Subscript $_S$ indicates phosphothioate linkage

iB: 3'-3' abasic moiety

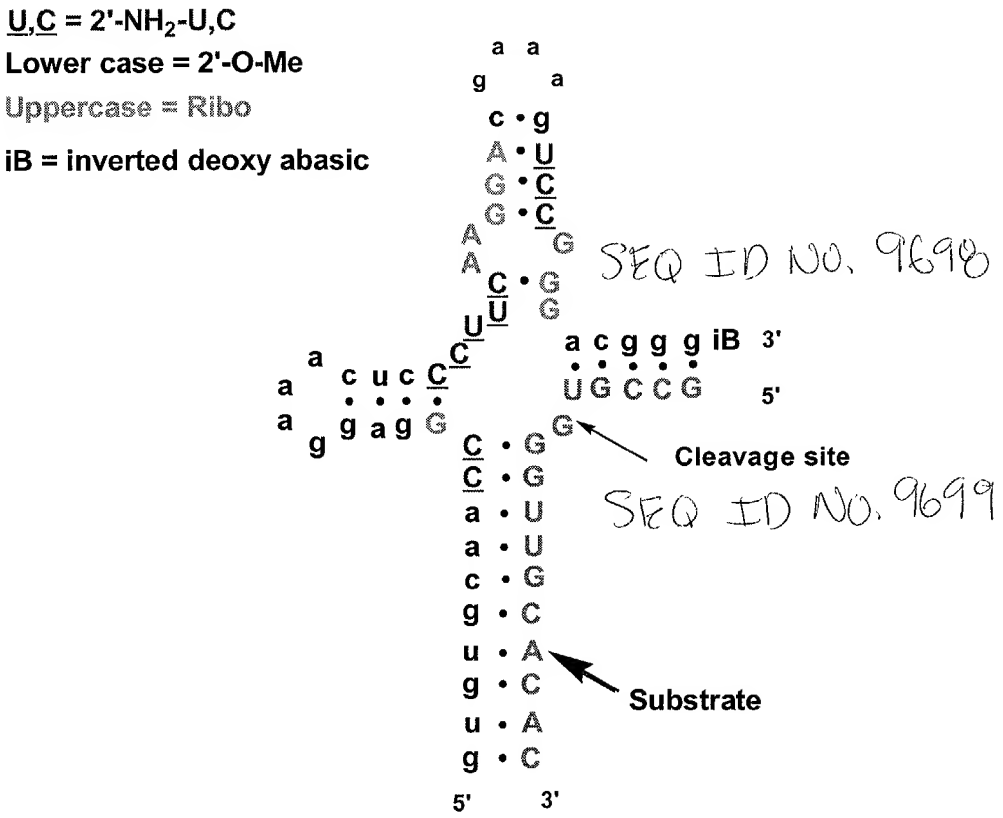
Y = U or C

G' can be G, ca, or caa

The gaaa tetraloop can be replaced by 18 atom polyethylene glycol (Spacer) or equivalent

All ribo G's in the Zinzyme can be replaced with 2'-*O-methyl* G, or combinations thereof

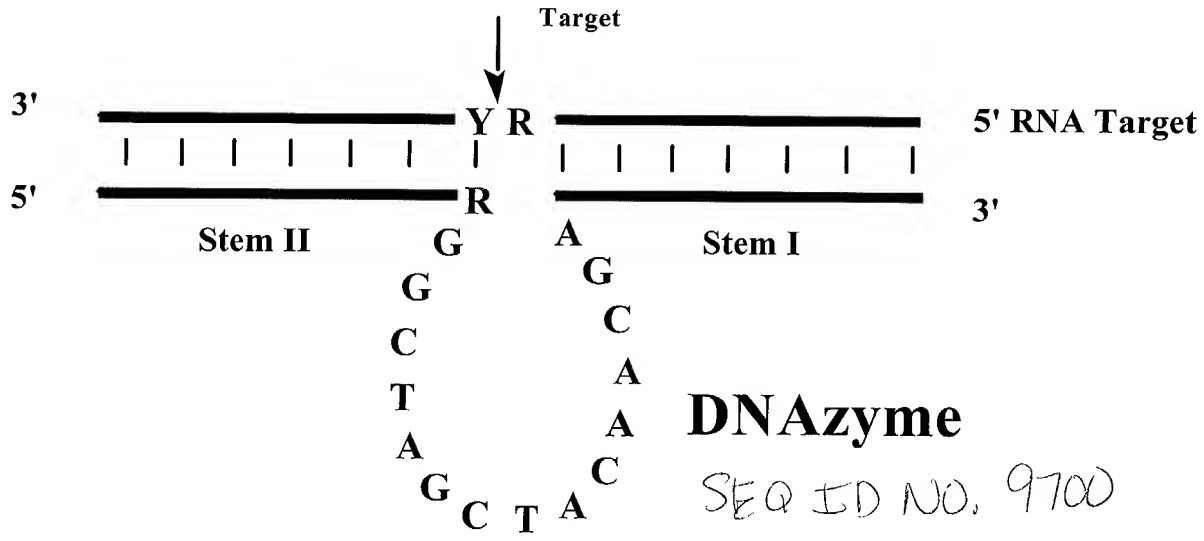
Figure 4. Chemically Stabilized Amberzyme Motif



The gaa loops may be replaced with loops of differing nucleic acid compositions, or with a linker, for example an 18 atom polyethylene glycol (Spacer) or equivalent.

Phosphorothioate linkages can be introduced, for example, at the 4 terminal 5'-internucleotide linkages, to increase nuclease stability.

Figure 5: DNAzyme Motif



Legend

Y = U or C
R = A or G

Figure 7: Secondary structure of the HCV 5'UTR

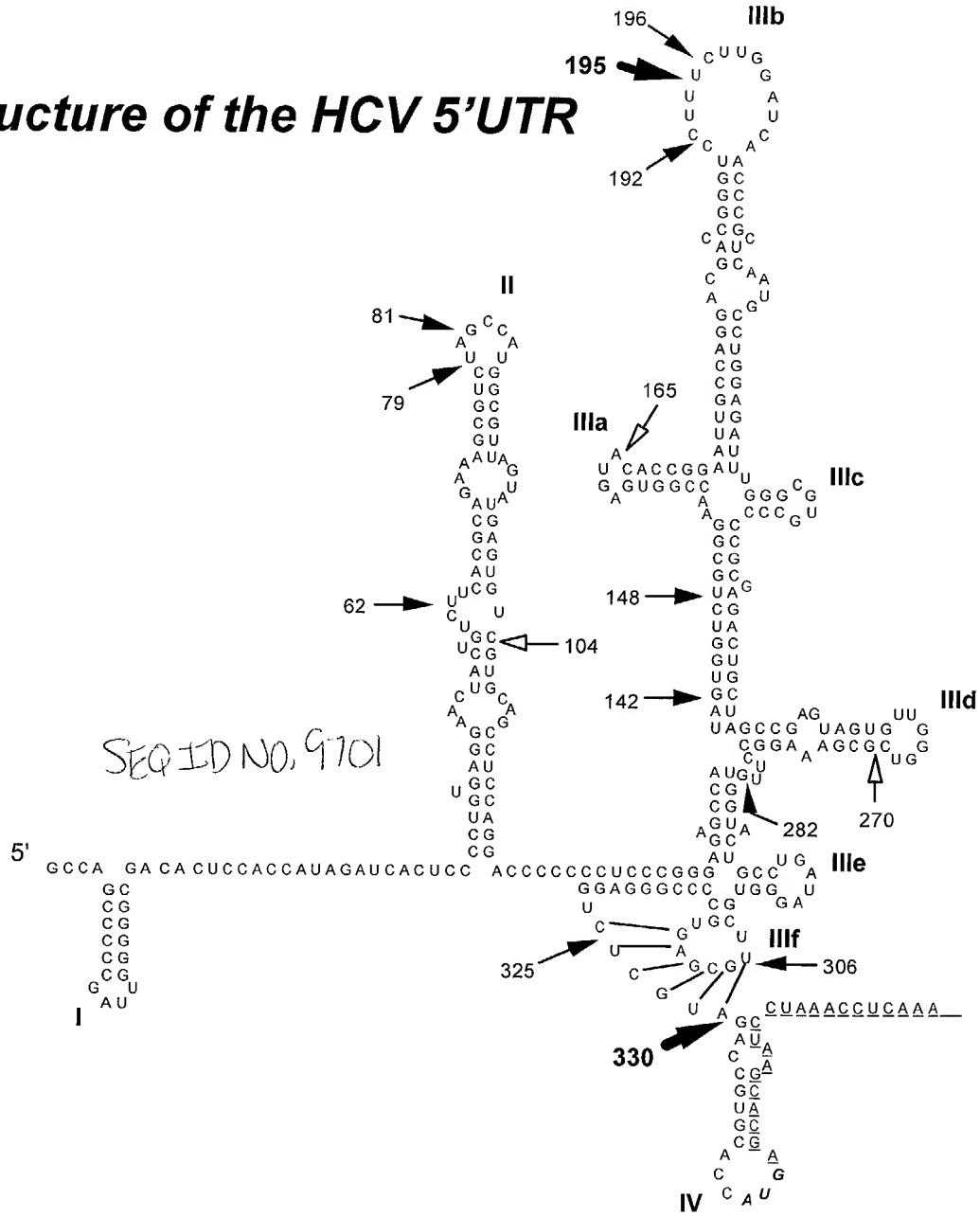
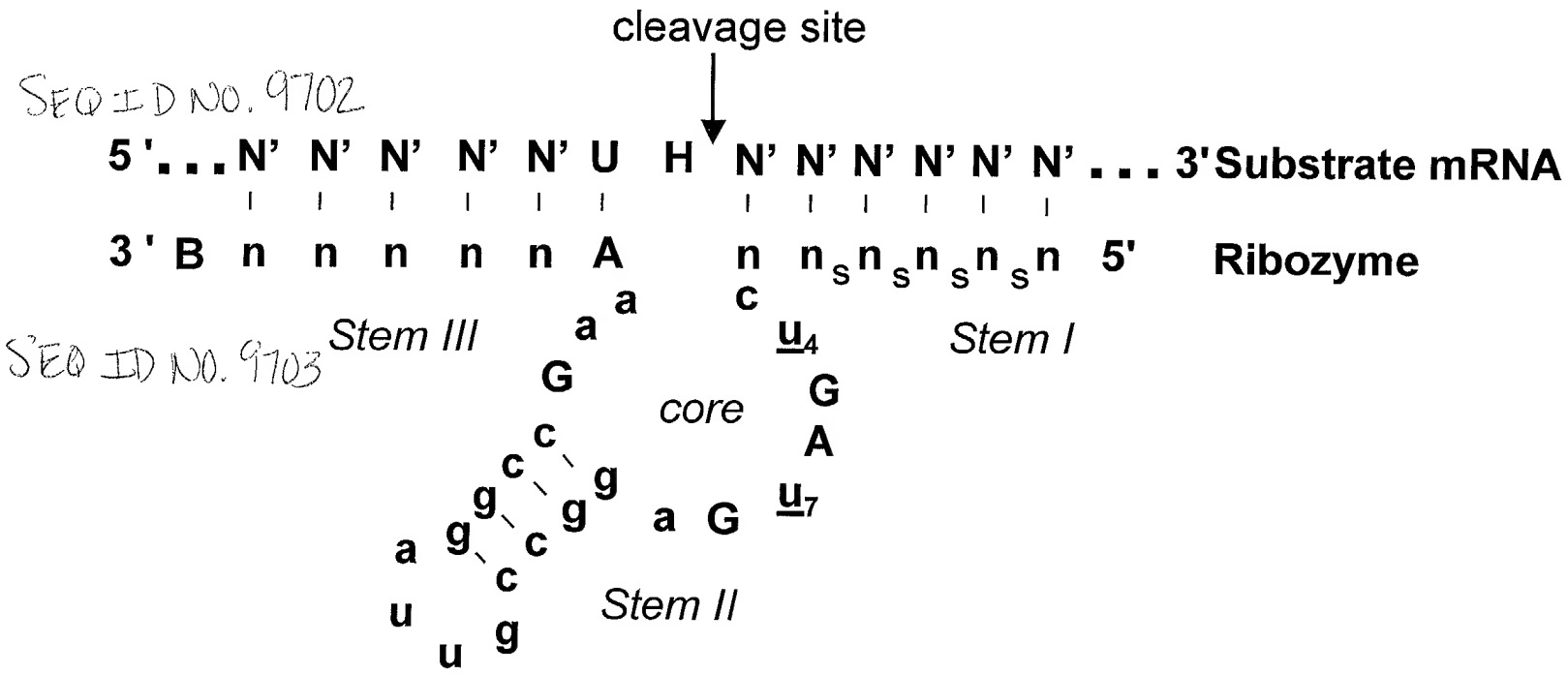


Figure 8: A Chemically Stabilized Enzymatic Nucleic Acid Molecule



UPPER CASE = RIBO nucleotide
lower case = 2'-O-methyl nucleitide
u = 2'-deoxy-2'-amino Uridine
s = phosphorothioate
B = inverted deoxyabasic moiety

Hepatitis Delta Virus (HDV) Ribozyme

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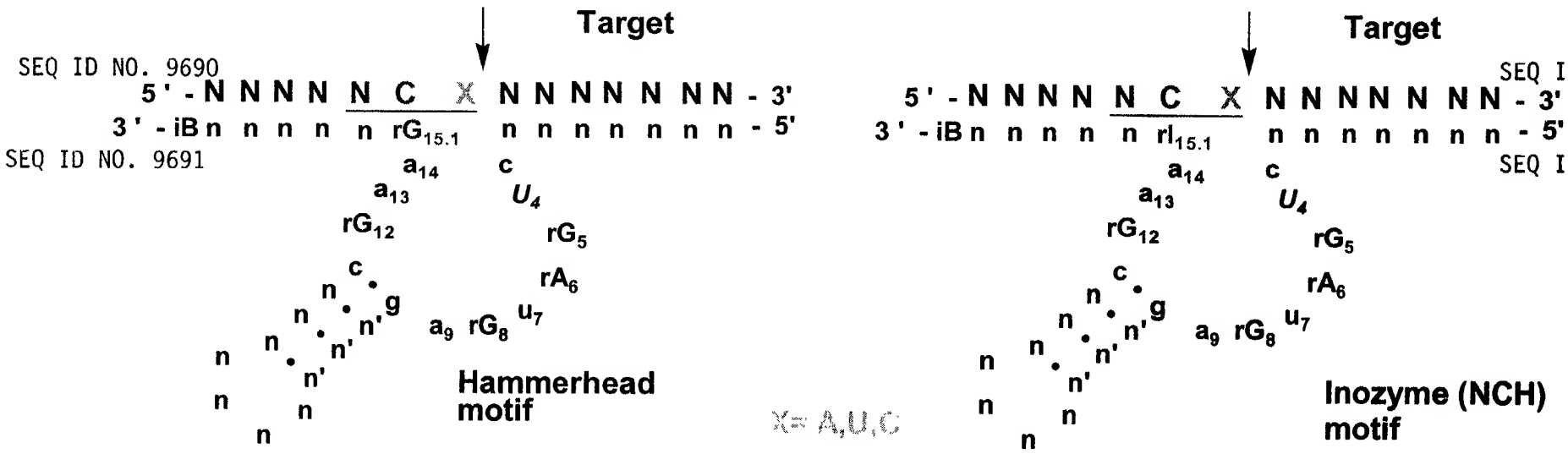
xxxix Perrotta, Anne T.; Been, Michael D.. Cleavage of oligoribonucleotides by a ribozyme derived from the hepatitis .delta. virus RNA sequence. Biochemistry (1992), 31(1), 16-21.

xl Perrotta, Anne T.; Been, Michael D.. A pseudoknot-like structure required for efficient self-cleavage of hepatitis delta virus RNA. Nature (London) (1991), 350(6317), 434-6.

xli Puttaraju, M.; Perrotta, Anne T.; Been, Michael D.. A circular trans-acting hepatitis delta virus ribozyme. Nucleic Acids Res. (1993), 21(18), 4253

Figure 1: Examples of Chemically Stabilized Enzymatic Nucleic Acid motifs



Lower case = 2'-*O*-Me

rN = ribonucleotide

U_4 = 2'-*C*-Allyl

rI = ribo Inosine modification

iB = inverted deoxy abasic

Figure 2: G-cleaver Motif

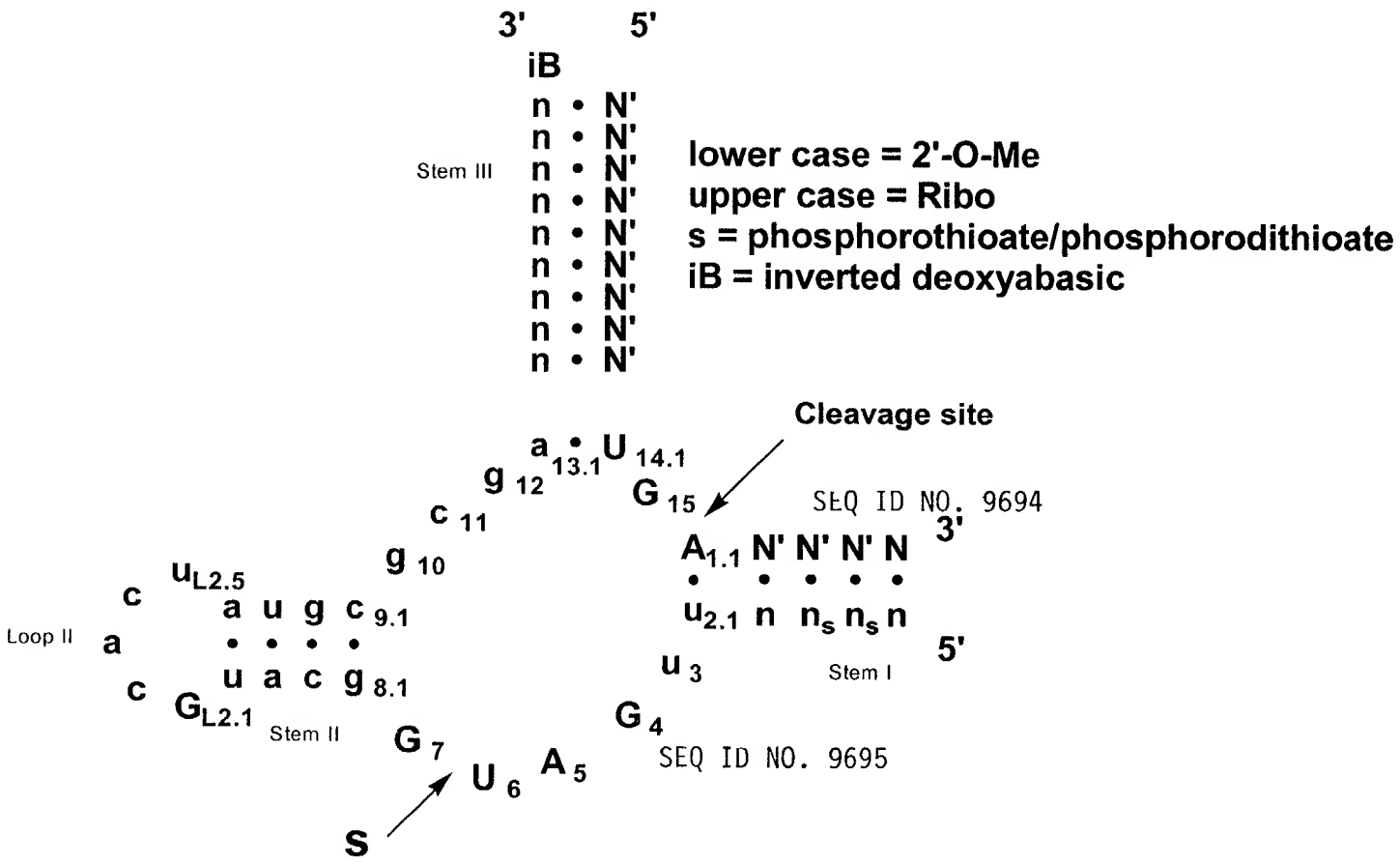
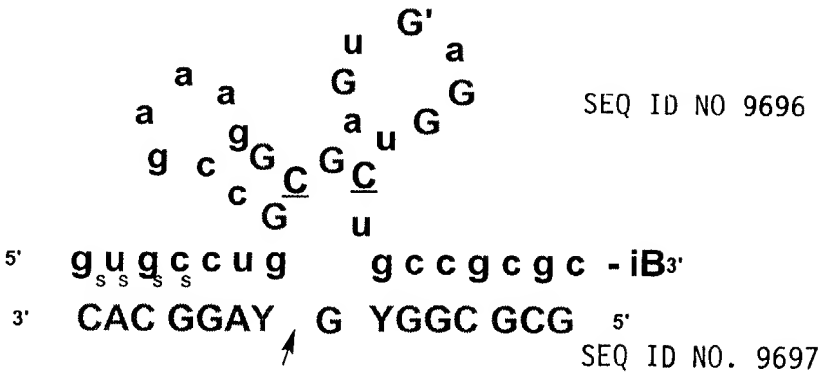


Figure 3: Chemically Stabilized Zinzyme Motif



SEQ ID NO 9696

SEQ ID NO. 9697

Legend

Uppercase indicates natural ribo residues

C indicates 2'-deoxy-2'-amino cytidine

Lowercase: 2'-*O*- Me

Subscript $_S$ indicates phosphothioate linkage

iB: 3'-3' abasic moiety

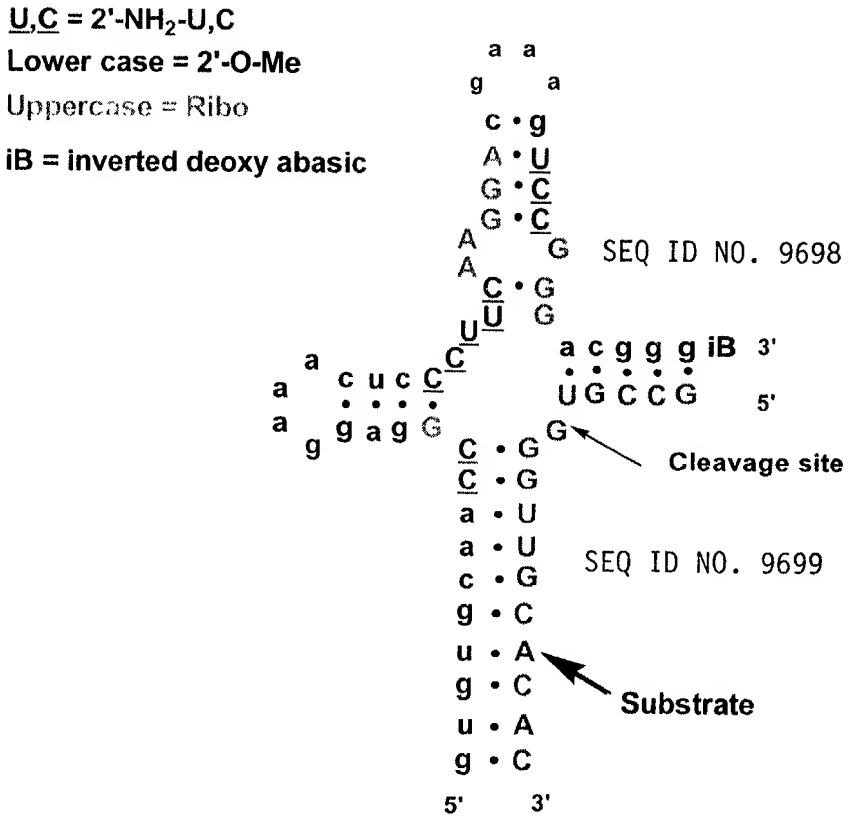
Y = U or C

G' can be G, ca, or caa

The gaaa tetraloop can be replaced by 18 atom polyethylene glycol (Spacer) or equivalent

All ribo G's in the Zinzyme can be replaced with 2'-*O-methyl* G, or combinations thereof

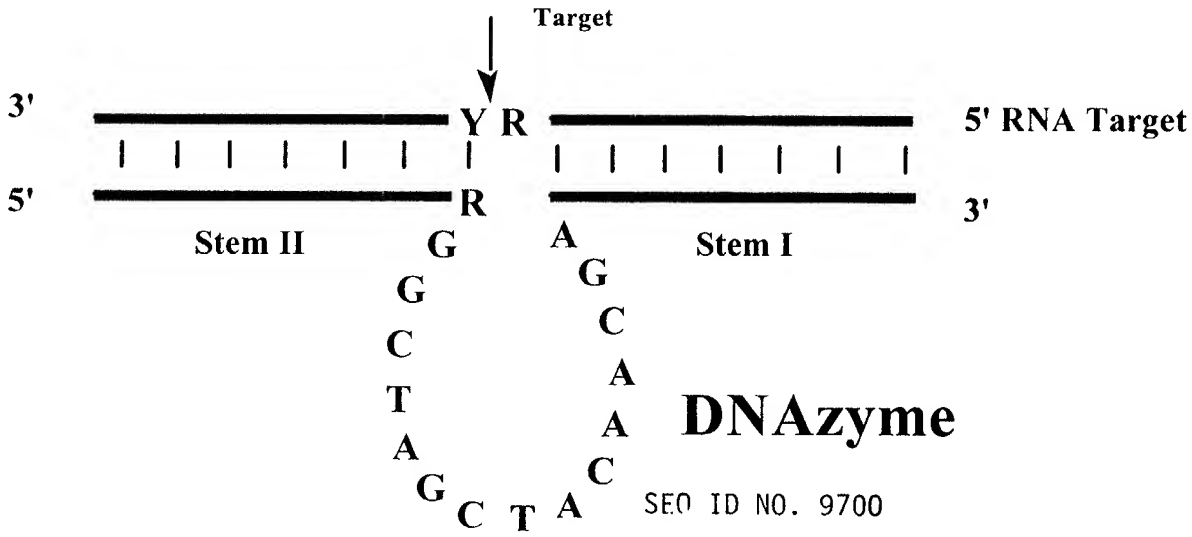
Figure 4. Chemically Stabilized Amberzyme Motif



The gaa loops may be replaced with loops of differing nucleic acid compositions, or with a linker, for example an 18 atom polyethylene glycol (Spacer) or equivalent.

Phosphorothioate linkages can be introduced, for example, at the 4 terminal 5'-internucleotide linkages, to increase nuclease stability.

Figure 5: DNAzyme Motif



Legend

Y = U or C
R = A or G

Figure 7: Secondary structure of the HCV 5'UTR

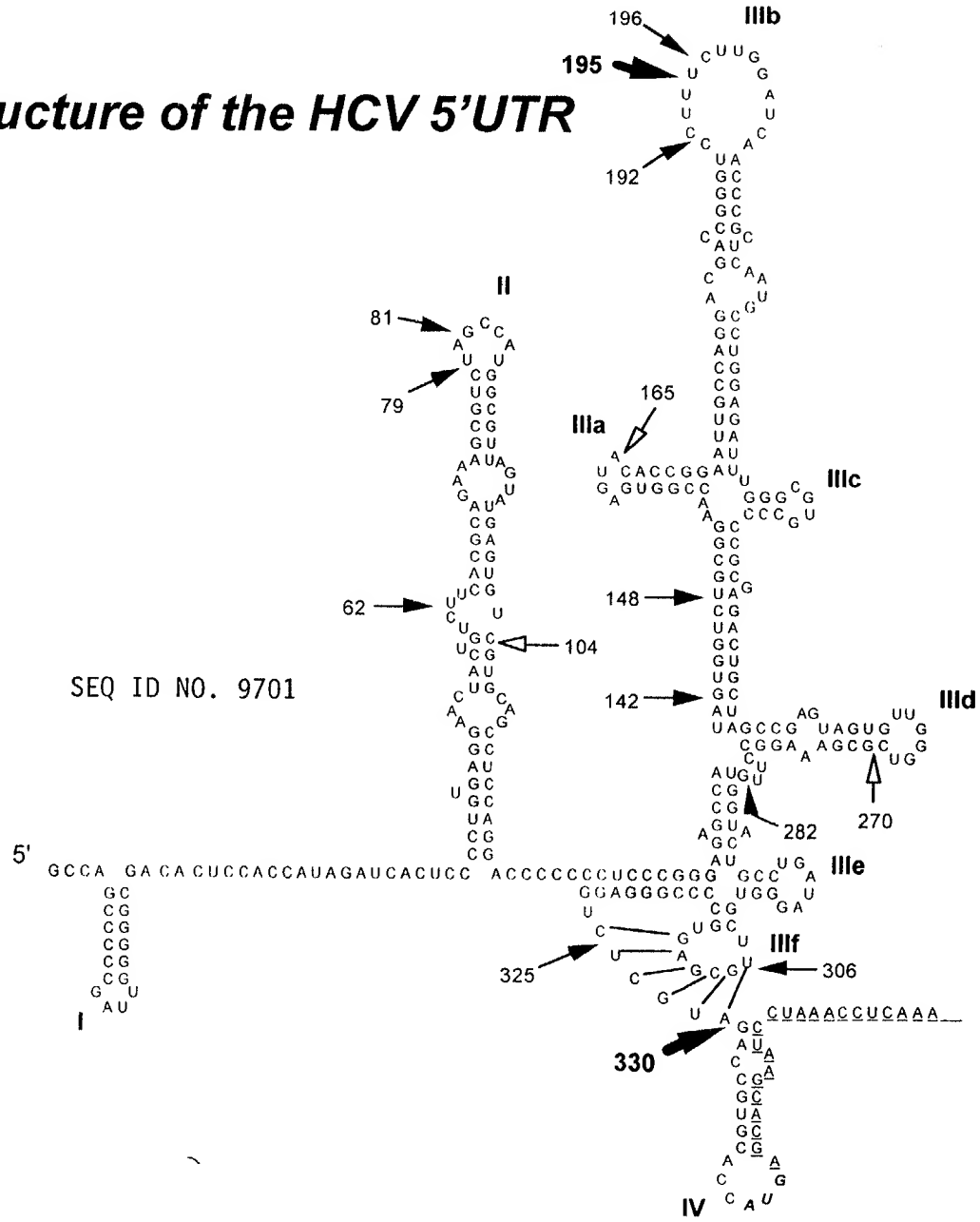
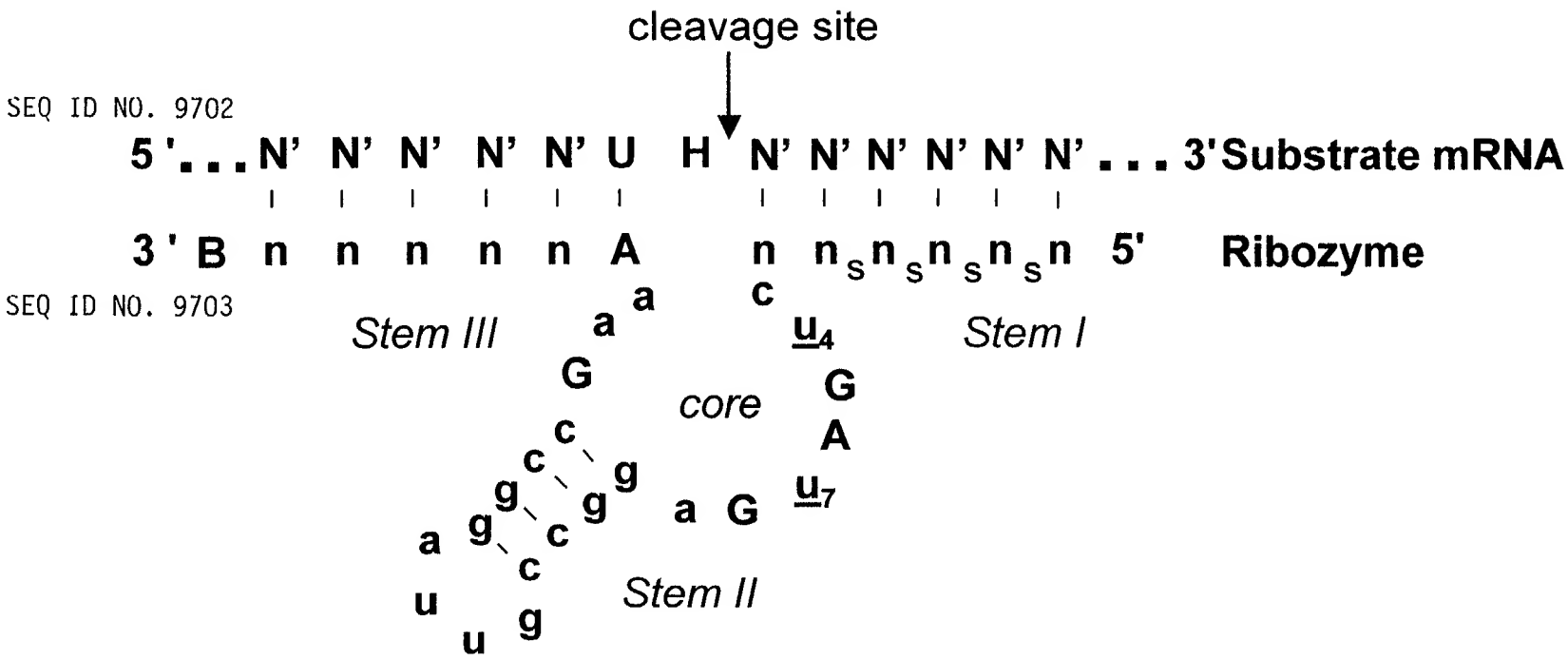


Figure 8: A Chemically Stabilized Enzymatic Nucleic Acid Molecule



UPPER CASE = RIBO nucleotide
lower case = 2'-O-methyl nucleitide
u = 2'-deoxy-2'-amino Uridine
s = phosphorothioate
B = inverted deoxyabasic moiety